bio-protocol Preprint

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Cell preparation and scRNA-seq

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An abbreviated version of this protocol was published in Science Advances in Aug 2020

Cellular diversity of the regenerating caudal fin

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Detailed protocol

Cell preparation and single-cell RNA-sequencing for zebrafish caudal fin tissue

I. Buffer preparations

1. One day before sample collection, thaw Accumax™ (Innovative Cell Technologies, Inc., CA) in 4˚C overnight

II. Tissue collection

i. Uninjured fin tissue collection

1. Anesthetize certain number of zebrafish as designed with 0.001% Tricaine (MS222) in fish water in a facility-approved container.
2. Move anesthetized fish on to a clean petri dish.
3. Use a razor/scalpel blade to cut at the middle of the caudal fin perpendicularly to the anterior-posterior axis
4. Transfer 2-3 segments at the anterior end of the removed fin tissue to a 1.5mL Eppendorf tube containing 1x Dulbecco's phosphate-buffered saline (DPBS) on ice.

ii. Regenerating fin tissue collection

1. To collect X day-post-amputation (dpa) tissues: Time the experiment to perform first time fin amputation on the X days before collection of X day-post-amputation (dpa). Anesthetize certain number of zebrafish as described above.
2. Use a razor/scalpel blade to cut at the middle of the caudal fin perpendicularly to the anterior-posterior axis.
3. Put fish back to circulating water system. Let them recover for X days.
4. On the day of collection, cut at the previously made cutting plane to obtain the regenerating tissue, and transfer to a 1.5mL Eppendorf tube containing 1x DPBS on ice.

III. Cell dissociation

1. Carefully replace 1x DPBS with 1mL thawed Accumax™
2. Incubate with rotation at room temperature for 1h.
3. During incubation, gently pipette the sample with wide-pore tips several times to help with dissociation.
4. Filter dissociated sample with 40µm cell strainers (for small sample size, we recommend using pipette tip strainers or home-made version with nylon mesh fixed onto 1000µL pipette tips).
5. Spin down at 500xg for 3min at 4˚C and wash the sample with 1mL cold 1xDPBS
6. Spin down at 500xg for 3min at 4˚C and resuspend with 100µL 1xDPBS-0.04% BSA
7. Count cells and adjust cell concentration depending on targeted cell recovery from Chromium controller.

IV. Library construction

1. Follow the instruction of the Chromium Single Cell Gene Expression Solution 3' v2 (10xGenomics) for library construction from cell suspensions.

* For data analysis post initial processing using cellranger pipeline, please see aba2084.r.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Hou, Y. , Zhao, G. and Wang, T. (2021). Cell preparation and scRNA-seq. Bio-protocol Preprint. bio-protocol.org/prep1036.
2. Hou, Y., Lee, H. J., Chen, Y., Ge, J., Osman, F. O. I., McAdow, A. R., Mokalled, M. H., Johnson, S. L., Zhao, G. and Wang, T.(2020). Cellular diversity of the regenerating caudal fin. Science Advances 6(33). DOI: 10.1126/sciadv.aba2084